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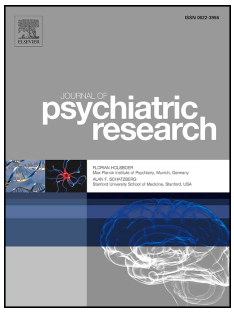

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A Meta-Analysis of Cytokine Concentrations in Eating Disorders

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Abstract

Cytokines are signalling molecules, which play an important role in both immune system function and brain development and function, and subsequently mental states and behaviour. Cytokines have been implicated in eating disorders (EDs) due to their role in psychological health, body weight and appetite regulation. This meta-analysis examined cross-sectional and longitudinal studies measuring concentrations of cytokines in individuals with EDs. Using PRISMA guidelines, we systematically reviewed relevant articles in PubMed, Web of Science, and MEDLINE. Random-effects meta-analyses were conducted for interleukin (IL)-1β, IL-6, transforming growth factor (TGF)-β, and tumor necrosis factor (TNF)-α, independently, firstly with all EDs combined and then stratified by ED diagnosis. Twenty-five studies were included: serum/plasma cytokine concentrations were measured in people with anorexia nervosa (AN) in 23 studies and bulimia nervosa (BN) in 4 studies. TNF-α and IL-6 were elevated in ED participants compared to healthy controls (HCs). Specifically, this pattern was seen only when comparing AN participants to HCs. Concentrations of these cytokines did not differ between people with BN and HCs. IL-1β and TGF-β did not differ between HCs and any ED group. Therefore, AN seems to be associated with elevated concentrations of TNF-α and IL-6. Considering the role of cytokines in appetite, mood regulation, and anxiety, these pro-inflammatory cytokines could be a potential future drug target helping people with AN, not only with weight gain, but also with various coexisting psychological problems. Future studies should consider confounding factors that affect cytokine concentrations and report ED-relevant clinical characteristics.

Keywords: eating disorders, cytokines, anorexia nervosa, bulimia nervosa, inflammation

1. Introduction

Eating disorders (EDs) are serious mental illnesses characterised by pathological eating and weight control behaviours, and body image disturbances. More specifically, anorexia nervosa (AN) involves food restriction and weight-control behaviours resulting in severe weight loss. Both bulimia nervosa (BN) and binge eating disorder (BED) are characterised by frequent bingeing, with BN also involving inappropriate compensatory behaviours, which are not seen in those with BED (American Psychiatric Association, 2013). It is estimated that approximately 20 million people within the European Union have an ED (Schmidt et al., 2016). However, the current available treatments for EDs are limited. For example, with respect to psychopharmacological treatment options, medications are limited to fluoxetine for BN and lisdexamfetamine for BED, which are only approved in certain countries (Himmerich and Treasure, 2018). Ultimately, just over half of individuals with BN and AN treated in specialist ED services make a full recovery (Smink et al., 2013; Steinhausen, 2002; Steinhausen and Weber, 2009), underscoring the need for a greater understanding of ED pathophysiology and for novel alternative treatment strategies.

Genetic, neurobiological, gastrointestinal, neuroendocrinological and immunological mechanisms have been implicated in the development and maintenance of EDs (Klein and Walsh, 2004; Slotwinska and Slotwinski, 2017). An immunological component, strongly interrelated to both the neuroendocrine and nervous systems, are cytokines. Cytokines have been shown to be altered in people with EDs compared to healthy individuals (Corcos et al., 2003), and are of particular current interest in EDs as genome wide association studies have identified significant genome-wide loci associated with AN that are closely linked with immune functioning and cytokine signalling (Duncan et al., 2017). Cytokines play a role as soluble intercellular signalling proteins with particular importance in the immune system. They are produced by a range of cells, including microglia and astrocytes, in both the brain and in the periphery (Lichtblau et al., 2013). There is no firm and generally accepted categorisation of cytokines (Cavaillon, 2001). However, functionally relevant groupings can be used: for example, pro-inflammatory cytokines e.g., tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) & IL-6; anti-inflammatory cytokines e.g., IL-10; and chemokines e.g., CXC chemokine ligand (Zhang and An, 2007).

Cytokines produced in the body's periphery can access the brain via humoral, neural and cellular pathways (see Capuron and Miller, 2011, for a review) and thus, have an effect on mental state, including learning, memory, affect, and behaviour through several pathophysiological mechanisms (Kelley et al., 2003; Yirmiya and Goshen, 2011). These mechanisms include an influence on the metabolism and signal transduction of neurotransmitters, modulation of neuroendocrine systems such as the hypothalamus-pituitary-adrenal (HPA) axis, induction of the release of hormones involved in feeding and appetite, and an impact on neural plasticity and neurogenesis (Capuron and Miller, 2011; Wong and Pinkney, 2004). Alterations in these biological systems may be particularly pertinent given that some of these have been linked to the pathophysiology of EDs (Klein and Walsh, 2004).

In recent years, evidence has shown that cytokines play an important role in mental health and the pathophysiology of mental disorders, including disorders which are highly comorbid with EDs such as depression (Dowlati et al., 2010; Lichtblau et al., 2013), anxiety disorders (Baldwin et al., 2017; Quagliato and Nardi, 2017), post-traumatic stress disorder (Hussein et al., 2017; Waheed et al., 2018), and sleep disorders (Weschenfelder et al., 2012). Cytokines have also been linked with body weight and its regulation (Fonseka et al., 2016); for example, plasma levels of pro-inflammatory cytokine IL-6 have been shown to correlate positively with body mass index (BMI) (Himmerich et al., 2006; Schmidt et al., 2015). Additionally, cytokines are involved in the regulation of food intake (Himmerich and Sheldrick, 2010) and appetite (Andréasson et al., 2007; Dent et al., 2012), which may be due to interactions with orexigenic and anorexigenic signals (Wong and Pinkney, 2004). Given the involvement of cytokines in psychological health, weight, and regulation of feeding behaviour and appetite, this provides a rationale for considering the role of cytokines in EDs.

Over the past two decades, several reviews have considered the role of cytokines in EDs (Brown et al., 2008; Corcos et al., 2003; Holden and Pakula, 1996; Marcos, 1997; Slotwinska and Slotwinski, 2017). More recently, a meta-analysis has shown circulating concentrations of pro-inflammatory cytokines TNF-α, IL-1β & IL-6 to be elevated in people with AN, in comparison to healthy controls (HCs) and the concentration of these cytokines did not change with weight gain (Solmi et al., 2015). However, research in other EDs is mixed (Corcos et al., 2003) and has not been recently systematically collated. Therefore, the aim of this meta-analysis was to synthesise studies investigating cytokine concentrations in individuals with EDs, both in comparison to healthy individuals and longitudinally, and to update the previously described meta-analysis on cytokines in AN (Solmi et al., 2015). In line with this, the key research questions were: (i) do cytokine concentrations differ between people diagnosed with an ED and healthy individuals; and (ii) in ED participants, do cytokine concentrations change as a function of weight gain and/or symptom improvement?

2. Material and methods

This meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009). We used the Newcastle-Ottowa Scale (NOS) (Wells et al., 2015) adapted for cross-sectional studies (see Supplementary File 1) to determine the quality of included studies. The NOS is a widely used quality assessment tool for non-randomised studies of multiple designs.

2.1. Literature search

Three electronic databases (PubMed, ISI Web of Science Core Collection, and MEDLINE via Ovid SP) were searched from inception until 4th May 2018, using the following keywords, which were mapped to Medical Subject Headings with the Explode function where possible: eating disorder*, anorexia nervosa, bulimi*, binge eat* in combination with cytokine*, chemokine*, inflammat*, interleukin, interferon, IFN, tumor necrosis factor, TNF, transforming growth factor, TGF. These searches were supplemented by internet searches, hand-searches of reference lists of potentially relevant papers and reviews, and citation tracking in Google Scholar.

2.2. Inclusion/exclusion criteria

Studies in any language of any study design that assessed cytokine concentrations in the serum, plasma or cerebrospinal fluid (CSF) of individuals with a Diagnostic and Statistical Manual of Mental Disorders (DSM) (American Psychiatric Association, 1980; American Psychiatric Association, 2000; American Psychiatric Association, 2013) or International Statistical Classification of Diseases (ICD) (World Health Organization, 1992) diagnosis of an ED were eligible for inclusion. Publications were included if they reported cross-sectional comparisons of cytokine concentrations between ED groups and HCs or longitudinal assessments. Longitudinal studies were included if cytokine concentrations were measured at a minimum of two time-points, and BMI or ED symptoms were also assessed at both time points.

Studies were excluded if: i) they did not report group comparisons *or* longitudinal measurements of cytokine concentrations; ii) participants had an organic cause for their disordered eating e.g., cancer, immunological conditions, genetic disorder, etc.; iii) the sample was comprised of animals; or iv) they measured cytokine production or genetic expression but did not assess cytokine concentrations. Review articles, meta-analyses, conference proceedings/abstracts, book chapters, and unpublished theses were also not included.

2.3. Search selection

Titles and abstracts of retrieved publications were imported into EndNote. Duplicates were removed, and papers deemed highly unlikely to be relevant were disregarded. Full-text versions of the remaining articles were then obtained and screened according to the pre-specified eligibility criteria described above. All papers that did not meet the inclusion criteria were excluded, with the reasons documented (see Figure 1). The entire search process was conducted independently by two reviewers (B.D. and S.B.) and disagreements at the final stage were resolved by consensus.

2.4. Data extraction

The principal reviewer (B.D.) extracted data from all included studies into an electronic summary table, which was then checked by another reviewer (S.B.). Information collected related to: i) sample characteristics, including sample size, demographics, diagnostic criteria and clinical characteristics (e.g. illness duration, BMI), and medication status; and ii) parameters of interest, measurement methods, and concentrations of cytokines. Authors were contacted if the required data were not available in the publication.

2.5. Summary measure

The principle outcome measure was cytokine concentrations (pg/ml or ng/ml).

2.6. Synthesis of data

Individual meta-analyses were performed for each cytokine (with 2 or more available studies; The Cochrane Collaboration, 2011) for all EDs combined and then for each ED separately. For studies that included more than one diagnostic group (e.g. AN and BN participants) or AN subtype, the means and standard deviations for each group were pooled for the combined EDs and/or AN meta-analyses. Reported standard error means were converted to SDs. The required data were not available to conduct meta-analyses on longitudinal measurements of cytokines, controlling for within-subject correlations.

2.7. Statistical analysis

All meta-analyses were conducted in Stata/SE 15.0 (StataCorp, 2017) using the 'metan' command. The standardised mean difference (SMD) was used as the summary statistic, which expresses the size of the effect in each study (EDs vs. HCs) relative to the variability observed in that study. SMD is used in meta-analyses when included studies assess the same outcome but measure it in a variety of ways and therefore it is necessary to standardise the results of the studies before they are combined (The Cochrane Collaboration, 2011). For all meta-analyses, a random effects model was specified using the DerSimonian & Laird method (DerSimonian and Laird, 1986) and SMDs were pooled using Hedges method (Hedges, 1981). The random effects model assumes both within-group variability and between-study heterogeneity. Positive SMDs were indicative of higher cytokine concentrations in the ED compared to the HC participants. A p-value <0.05 indicated a significant difference between the ED and HC groups.

Between study heterogeneity was assessed by calculating Higgins I^2 (Higgins et al., 2003) based on Cochran's Q indexes. I^2 measures the percentage of total variation across studies due to heterogeneity. Moderate (50%) to high (75% to 100%) heterogeneity was suspected between studies, and for this reason a random effects meta-analysis was used in all cases (Higgins et al., 2003). Significant between-study heterogeneity was further explored using sub-group analyses and/or meta-regressions using the STATA 'metareg' command. The meta-regressions investigated the effect of age, BMI and illness duration on the SMD in cytokines between ED and HC groups. Subgroup analyses of the AN meta-analyses were stratified by AN subtype (restrictive [AN-R] or binge-purge [AN-BP]).

Publication bias was assessed using the Duval and Tweedie trim and fill method (Duval and Tweedie, 2000), which identifies and adjusts for funnel plot asymmetry, and Eggers test (Egger et al., 1997) for small study effects.

INSERT FIGURE 1 HERE

3. Results

3.1. Characteristics of included studies and participants

We identified 25 studies (ED n=632, HC n=487), conducted in 9 countries, which met the inclusion criteria for a quantitative analysis and for which the required data were available (see Figure 1 for PRISMA flow diagram). Study and sample characteristics are presented in Table 1. Two studies included multiple ED subgroups (Ahrén-Moonga et al., 2011; Brambilla et al., 1998). Twenty-three studies reported cross-sectional comparisons in cytokine concentration between participants with AN (n=538) and HCs, identifying four new studies not included in the previous meta-analysis (Solmi et al., 2015). Within these, 12 reported sub-type information: all assessed cytokine concentrations in participants with AN-R and 3 studies additionally reported on participants with AN-BP separately. Four studies compared cytokine concentrations in BN participants (n=75) to HCs. No studies assessed cytokine concentrations in patients with BED or other EDs.

The mean age of participants (reported in n=19 studies) with EDs and HCs was 21.39 ± 4.10 and 22.62 ± 5.18, respectively. All studies only included female participants. The mean BMI (reported in n=20 studies) of ED participants was 15.63 ± 1.83 kg/m^2 (AN participants, n=19 studies: 15.15 ± 1.17 kg/m^2, BN participants, n=2 studies: 21.25 ± 2.19 kg/m^2) and of HCs was 21.32 ± 1.98 kg/m^2, respectively. Mean illness duration for ED participants, reported in 8 studies, was 3.66 ± 2.59 years. ED diagnosis was based on the DSM-IV (n=24) (American Psychiatric Association, 2000) or the DSM-III (n=1) (American Psychiatric Association, 1980). Medication usage of ED participants was reported in 14 studies, of which participants in 11 studies were confirmed medication-free at assessment (Allende et al., 1998; Brambilla et al., 1998; Brambilla et al., 2001; Dolezalova et al., 2007; Karczewska-Kupczewska et al., 2013; Karczewska-Kupczewska et al., 2012; Nagata et al., 2006; Nakai et al., 1999; Nakai et al., 2000; Pomeroy et al., 1994; Shimizu et al., 2005). In two studies (Ahrén-Moonga et al., 2011; Nogueira et al., 2010), n=26 were reported to be taking antidepressants, neuroleptics, anxiolytics and/or sedatives, and in the remaining study, participants were not taking medication known to affect nutritional or bone status (Ostrowska et al., 2016).

Cytokines included in the meta-analyses were IL-1β, IL-6, TGF-β and TNF-α. Other cytokines (IFN-γ, IL-1, IL-2, Il-4, IL-5, IL-7, IL-10, macrophage inhibitory cytokine-1) were also measured in eligible studies, however, the data were not available for two or more studies and could not be included in a meta-analysis. Seventeen studies measured cytokine concentrations in serum, 8 in plasma and none in CSF. Measurement methods included immuno-assays (22 studies: enzyme immune-assay n=18, radio immune-assay n=2, undefined n=2) or bioassay (1 study). The remaining studies did not provide sufficient information to classify their measurement methods.

The quality ratings for each study are presented in Supplementary File 1. Few studies ensured representativeness of sample through their sampling method (n=6) or provided justification for their sample size (n=1). Most studies accounted for age as a confounding factor (n=21), however, the majority of studies did not control for additional important confounding factors (e.g., smoking and BMI). Thirteen studies used a validated method to measure outcome, with the remaining studies using a non-validated measurement method that was described in sufficient detail. In all studies, the statistical test used was clearly described and appropriate.

INSERT TABLE 1 HERE

3.2. Meta-analysis results

Results are summarised in Table 2 and forest plots not presented here can be seen in Supplementary File 2.

3.2.1. Across all EDs

Pro-inflammatory cytokines. IL-1β was measured in 7 studies including 205 ED participants and 102 HCs (Allende et al., 1998; Brambilla et al., 1998; Brambilla et al., 2001; Nogueira et al., 2010; Ostrowska et al., 2015; Vaisman et al., 2004; Yasuhara et al., 2007). The concentration of IL-1β did not differ between groups (SMD=0.77; 95% CI -0.13, 1.66; *p*=0.093).

Thirteen studies measured IL-6 (Ahrén-Moonga et al., 2011; Brambilla et al., 1998; Brambilla et al., 2001; Corcos et al., 2001; Dolezalova et al., 2007; Karczewska-Kupczewska et al., 2013; Misra et al., 2006; Nagata et al., 2006; Ostrowska et al., 2015; Pomeroy et al., 1994; Terra et al., 2013; Víctor et al., 2015; Yasuhara et al., 2007) and found concentrations of IL-6 to be significantly higher in participants with EDs (n=331) than HCs (n=258) (SMD=0.53; 95% CI 0.19, 0.87; *p*=0.002).

Across 18 studies (Agnello et al., 2012; Ahrén-Moonga et al., 2011; Allende et al., 1998; Brambilla et al., 1998; Brambilla et al., 2001; Corcos et al., 2001; Jiskra et al., 2000; Karczewska-Kupczewska et al., 2012; Krizova et al., 2008; Krizova et al., 2002; Nakai et al., 1999; Nakai et al., 2000; Nogueira et al., 2010; Ostrowska et al., 2015; Shimizu et al., 2005; Vaisman et al., 2004; Víctor et al., 2015; Yasuhara et al., 2007), there were significantly higher concentrations of TNF-α in ED participants (n=454) compared to HCs (n=344) (SMD=0.56; 95% CI 0.17, 0.94; *p*=0.005).

Other cytokines. Three studies that measured TGF-β (Corcos et al., 2001; Ostrowska et al., 2016; Pomeroy et al., 1994) suggested lower concentrations in ED participants (n=105; consists of exclusively AN participants) compared to HCs (n=51), though this difference did not reach statistical significance (SMD=-0.59; 95% CI -2.37, 1.20; *p*=0.518).

3.2.2. Anorexia nervosa

Pro-inflammatory cytokines. IL-1β measurements were made in 162 AN participants and 102 HCs from 7 studies (Allende et al., 1998; Brambilla et al., 1998; Brambilla et al., 2001; Nogueira et al., 2010; Ostrowska et al., 2015; Vaisman et al., 2004; Yasuhara et al., 2007), extending the previous meta-analysis (Solmi et al., 2015) by 3 studies (additional 77 AN participants and 35 HCs). Between-group differences in IL-1β did not reach statistical significance in AN (SMD=0.78; 95% CI -0.17, 1.72; *p*=0.110), nor in subgroup analyses of AN-BP participants (SMD=1.13; 95% CI -0.30, 2.57; *p*=0.122) (Brambilla et al., 1998; Brambilla et al., 2001; Nogueira et al., 2010). Participants with AN-R showed significantly higher levels of IL-1β than HCs (SMD=0.49; 95% CI 0.08, 0.89; *p*=0.018) (Brambilla et al., 1998; Brambilla et al., 2001; Nogueira et al., 2010; Yasuhara et al., 2007).

Measurements of IL-6 were extracted from 12 studies, consisting of 276 AN participants and 244 HCs (Ahrén-Moonga et al., 2011; Brambilla et al., 1998; Brambilla et al., 2001; Corcos et al., 2001; Dolezalova et al., 2007; Karczewska-Kupczewska et al., 2013; Misra et al., 2006; Ostrowska et al., 2015; Pomeroy et al., 1994; Terra et al., 2013; Víctor et al., 2015; Yasuhara et al., 2007); including an additional 94 AN and 64 HC participants (from 3 extra studies) compared to Solmi et al. (2015). There were significantly higher concentrations of IL-6 in AN participants compared to HCs (SMD=0.59; 95% CI 0.24, 0.94; *p*=0.001) (see Figure 2). Subgroup analyses found a trend towards higher levels of IL-6 in AN-R compared to HC participants, but this difference did not reach statistical significance (SMD=0.36; 95% CI -0.07, 0.78; *p*=0.105) (Brambilla et al., 1998; Brambilla et al., 2001; Karczewska-Kupczewska et al., 2013; Misra et al., 2006; Terra et al., 2013; Yasuhara et al., 2007).

INSERT FIGURE 2 HERE

Across 17 studies (Agnello et al., 2012; Ahrén-Moonga et al., 2011; Allende et al., 1998; Brambilla et al., 1998; Brambilla et al., 2001; Corcos et al., 2001; Jiskra et al., 2000; Karczewska-Kupczewska et al., 2012; Krizova et al., 2008; Krizova et al., 2002; Nakai et al., 1999; Nogueira et al., 2010; Ostrowska et al., 2015; Shimizu et al., 2005; Vaisman et al., 2004; Víctor et al., 2015; Yasuhara et al., 2007), TNF-α concentrations were significantly higher in AN participants (n=380) compared to HCs (n=324) (SMD=0.48; 95% CI 0.09, 0.87; *p*=0.015) (see Figure 3). This expands the sample of the previous meta-analysis (Solmi et al., 2015) by 145 AN participants and 106 HCs from 6 additional

studies. Subgroup analyses showed that between-group differences for AN-R (Brambilla et al., 1998; Brambilla et al., 2001; Jiskra et al., 2000; Karczewska-Kupczewska et al., 2012; Krizova et al., 2002; Nogueira et al., 2010; Shimizu et al., 2005; Yasuhara et al., 2007) and AN-BP (Brambilla et al., 1998; Brambilla et al., 2001; Nogueira et al., 2010) compared to HCs did not reach statistical significance (AN-R: SMD=0.12; 95% CI -0.20, 0.44; p=0.470; AN-BP: SMD=-0.50; 95% CI -1.31, 0.30; p=0.221).

INSERT FIGURE 3 HERE

Other cytokines. See analysis for TGF-β in Section 3.3.1. This analysis included a recent study (Ostrowska et al., 2016) unavailable for the previous meta-analysis (Solmi et al., 2015), which increased the AN sample by 60 participants.

3.2.3. Bulimia Nervosa

Pro-inflammatory cytokines. IL-6 concentrations were measured in 55 participants with BN and 52 HCs, taken from 3 studies (Ahrén-Moonga et al., 2011; Brambilla et al., 1998; Nagata et al., 2006). Between-group differences did not reach statistical significance (SMD=0.13; 95% CI -0.49, 0.76; p=0.67).

Concentrations of TNF-α were extracted from 3 studies (Ahrén-Moonga et al., 2011; Brambilla et al., 1998; Nakai et al., 2000), suggesting higher concentrations of TNF-α concentrations in BN participants (n=55) compared to HCs (n=58); however, this did not reach statistical significance (SMD=0.90; 95% CI -0.26, 2.06; p=0.13).

INSERT TABLE 2 HERE

3.2.4. Meta-regressions

The results of the meta-regressions are presented in Table 3. Age significantly contributed to the SMDs for IL-6 for the combined ED group and AN group, which may account for some of the observed heterogeneity. The remaining results were non-significant and BMI did not significantly contribute to the SMDs in any group.

Some meta-regressions were limited by the number of studies that could be included, particularly in relation to IL-1β, as only 6 studies were available. This violates the recommended guidelines of a requirement of 10 studies for a meta-regression (The Cochrane Collaboration, 2011). For this reason, illness duration could not be included in the meta-regressions as this limited the number of studies to n=5.

INSERT TABLE 3 HERE

3.2.5. Sensitivity analyses

Moderate to high heterogeneity (>50%) was observed in all meta-analyses conducted (except for AN-R IL-1β and TNF-α), as seen in Table 2. According to Egger's test for small study effects, there is presumed to be no publication bias, as all analyses were non-significant. The trim and fill method showed that there were missing studies in the analysis of TNF-α concentrations in BN and TGF-β in

AN. When the SMDs were re-estimated after adjusting for missing studies, they remained non-significant. All other analyses showed no evidence of missing data using the trim and fill method.

4. Discussion

4.1. Summary of findings

This is the first meta-analysis to investigate cytokine concentrations across all EDs. AN was most researched (n=23 studies), followed by fewer investigations of cytokine concentrations in BN (n=4 studies). No studies reported on cytokine concentrations in BED or other EDs. The only available cytokines to be included in meta-analyses were TNF-α, IL-6, IL-1β, and TGF-β. Generally, studies reported on a limited number of cytokines and several important cytokines are yet to be measured in ED samples; e.g., IL-17 is a key cytokine for immune response and seems to play a role in the development of other psychiatric disorders and their treatment (Borovcanin et al., 2012; Davami et al., 2016; Himmerich et al., 2011). This highlights the need for future studies to assess a broad range of cytokines. Moderate to high heterogeneity in most analyses may be accounted for by methodological issues, as discussed below (Section 4.4). The required data were not available to assess changes in cytokine concentrations longitudinally therefore, we were unable to investigate our second research question.

In whole-group analyses for EDs, both TNF-α and IL-6 concentrations were found to be elevated in comparison to HCs. In sub-group analyses, these differences were observed in the AN, but not in the BN group, suggesting that the findings in the combined ED group, may have been driven by the elevation in AN participants. Results in AN participants replicate those identified in the previous meta-analysis (Solmi et al., 2015). Both TNF-α and IL-6 are classed as pro-inflammatory cytokines involved in the acute-phase response and are secreted into the blood stream in response to an immunological challenge. TNF-α is produced by macrophages, natural killer cells and T cells and stimulates the release of other pro-inflammatory cytokines and neutrophils, and induces fever (Abbas et al., 2014). IL-6 is produced by macrophages, endothelial cells and T cells, and is involved in the proliferation of antibody-producing cells (Abbas et al., 2014).

Similar to the findings in Solmi et al. (2015), TGF-β, a multi-functional transforming growth factor, was not found to differ between ED and HC participants. However, this analysis was limited to three studies in AN participants and therefore it is premature to draw conclusions from these data. In addition, IL-1β, a pro-inflammatory acute-phase response protein, did not differ between ED, AN or BN participants and HCs. Including three additional studies in the current meta-analysis did not replicate the findings from the previous meta-analysis (Solmi et al., 2015), which found elevated levels of IL-1β in AN compared to HCs. However, in subgroup analyses, IL-1β was found to be elevated in participants with AN-R, but not AN-BP, compared to HCs, as seen in Solmi et al. (2015). The significant findings limited to this restrictive subgroup may make sense given that IL-1β is anorexigenic and findings from animal studies have implicated IL-1β in reduced food intake: more specifically, administration of IL-1β results in reduced meal size and meal duration, but not meal frequency, and reduced food-seeking behaviour (Plata-Salaman, 2001; Wong and Pinkney, 2004).

Meta-regressions suggest that age may have contributed to the SMDs observed for IL-6 for both the combined ED and AN groups. Variations in age may therefore account for some of the observed heterogeneity, highlighting the importance of including a range of ages in investigations of cytokine concentrations in EDs. When included as a covariate, BMI did not appear to be a major factor influencing cytokine concentrations in people with EDs. In line with this, when BMI was used as a covariate in an included study, significantly elevated levels of TNF-α and IL-6 were still observed in AN participants compared to HCs (Víctor et al., 2015).

4.2. Mechanisms of effect

There are several mechanisms that may account for the elevated pro-inflammatory cytokines observed in those with EDs compared to HCs. Two such factors will be briefly discussed: stress, and the gut microbiota.

Firstly, stress can induce the release of and increase production of pro-inflammatory cytokines (Ménard et al., 2017). This elevation has been found in a number of animal studies in which hyper-production of cytokines was induced by acute and chronic stress paradigms (Himmerich et al., 2013;

Krügel et al., 2014; Liu et al., 2012). The mechanism as to how stress leads to an increase of pro-inflammatory cytokine production remains unclear. Importantly, psychological stress has been shown to augment the production of cytokines in humans (Glaser and Kiecolt-Glaser, 2005; Steptoe et al., 2007). Therefore, the presence of depression and/or anxiety, which are both highly comorbid with EDs, may play a role in the observed elevated concentrations of TNF-α and IL-6 (Dowlati et al., 2010; Felger and Lotrich, 2013; Furtado and Katzman, 2015; Kim and Won, 2017). Few studies assessed levels of stress, depression or anxiety and therefore, this cannot be determined.

Secondly, bacteria from the gut microbiota can stimulate the production of cytokines. This occurs when bacterial determinants, e.g., the lipopolysaccharide (LPS) component of the bacteria's cell walls, bind to pattern recognition receptors, e.g., Toll-like receptor 4, on circulating monocytes and macrophages (Sherwin et al., 2016) and gut epithelial cells. It is likely that dysregulation of intestinal microbiota is associated with EDs, given that the profile of gut microbiota is determined by the host's diet and EDs are characterised by dysregulated food intake (Lam et al., 2017). Furthermore, in AN, starvation is thought to provoke a 'leaky gut' in which the intestinal epithelial barrier is broken down (Herpertz-Dahlmann et al., 2017). This leads to 'leaking' of bacteria and/or their components from the gut into circulation which is then thought to elicit an inflammatory response i.e. stimulating cytokine production (Herpertz-Dahlmann et al., 2017; Sherwin et al., 2016). Little research has considered the role of gut microbiota in BN or BED. However, gut microbiota and regulation of the "gut-brain axis" have been proposed to play a significant role in stress and other psychiatric disorders that are highly comorbid with BN and BED, such as depression and anxiety, via their influence on inflammatory cytokines. This suggests such factors could contribute to alterations in cytokine production in these EDs (Alam et al., 2017; Kelly et al., 2015).

Once cytokine alterations have occurred through these and/or other mechanisms, cytokines can affect brain function and development, and subsequently mental states and behaviour, through their influence on several systems (Capuron and Miller, 2011; Wong and Pinkney, 2004). Cytokines can influence the synthesis, release, and reuptake of relevant neurotransmitters, such as serotonin and dopamine (Felger and Lotrich, 2013). For example, cytokines have been shown to influence the synthesis of neurotransmitters by stimulating the production of Indoleamine 2,3 dioxygenase, which breaks down tryptophan, an essential amino acid. This depletion of tryptophan is thought to contribute to a reduced availability of serotonin (Capuron and Miller, 2011; Miller et al., 2009). In addition, pro-inflammatory cytokines have been shown to disrupt tetrahydrobiopterin (BH4), which is an enzyme that is an essential cofactor for enzymes involved in the synthesis of monoamine neurotransmitters, including dopamine and serotonin (Haroon et al., 2012; Miller et al., 2013). This may be particularly pertinent, given that dysregulated serotonin and dopamine have been implicated in EDs (Broft et al., 2011; Gauthier et al., 2014; Kaye et al., 2005; O'Hara et al., 2015). Furthermore, the direct action of cytokines on the brain can influence neuroendocrine functioning. For example, cytokine exposure has been shown to activate the HPA axis, potentially by inhibiting glucocorticoid receptors (Capuron and Miller, 2011; Pace and Miller, 2009). This is of importance given that hyperactivation of the HPA axis has been reliably observed in AN, and more mildly in BN patients, in the acute phase of illness (Lo Sauro et al., 2008).

4.3. Clinical implications

As we identified elevated concentrations of circulating TNF-α and IL-6 levels in people with AN compared to HCs, cytokines may represent a potential biomarker of AN. However, as cytokines have been found to be elevated in other psychiatric disorders (e.g., Baldwin et al., 2017; Dowlati et al., 2010; Dunjic-Kostic et al., 2013b; Passos et al., 2015) and inflammatory diseases (e.g., Aaltonen et al., 2012), cytokines may not be a specific biomarker for AN but rather a non-specific marker of overall illness severity or general treatment response.

Cytokines such as IL-6 and TNF-α have repeatedly been shown to reduce food intake in animals and humans after peripheral and central administration (Langhans and Hrupka, 1999; McCarthy, 2000). Therefore, it has been suggested that they contribute to the development of anorexia in various infectious, neoplastic and autoimmune diseases. This seems to be due to peripheral anorexigenic effects of cytokines, such as an increase in the production of leptin, a hormone produced by the

adipose tissues (Finck et al., 1998; Grunfeld et al., 1996; Kirchgessner et al., 1997), but also due to the anorectic effects of pro-inflammatory cytokines on appetite-regulating hormones such as histamine, alpha-melanocyte stimulating hormone, ghrelin, and neuropeptide Y (Himmerich and Sheldrick, 2010; Huang et al., 1999; Langhans and Hrupka, 1999; Sahu et al., 1988; Wong and Pinkney, 2004).

Additionally, as mentioned, pro-inflammatory cytokines have been implicated in the pathophysiology of depression (Dowlati et al., 2010; Lichtblau et al., 2013), anxiety (Baldwin et al., 2017), and disturbed sleep (Weschenfelder et al., 2012). Patients with AN often do not want medication that induce weight gain, although this is necessary from a medical perspective, but rather want help with anxiety, low mood and sleep disturbances (Himmerich et al., 2017). Cytokines could theoretically be drug targets for the treatment of AN that addresses both the medical necessity for weight gain and patients' priorities of coexisting psychological problems. There is currently very little research investigating the therapeutic effect of cytokine blockers in AN (e.g., Barber et al., 2003; Solmi et al., 2013). However, these biologics have been shown to reduce depressive symptoms in patients with inflammatory diseases such as psoriasis (Tyring et al., 2006), and antidepressant-like effects of TNF-α blockers have been seen in rats, using a chronic stress model for depression (Krügel et al., 2013). In addition, anti-TNF-α medication has been shown to lead to increases in body weight in those with chronic inflammatory diseases (Ouchi et al., 2011).

As seen in the included studies, some ED participants show similar cytokine concentrations to HCs (e.g., Agnello et al., 2012). Therefore, it is important to consider that only a subgroup of patients will present with an immunological basis to the disorder, displaying elevated cytokine concentrations compared to HCs and for these patients, cytokines may be a potential future treatment target (Himmerich and Treasure, 2018).

Taken together, a subgroup of individuals with AN may benefit from treatment targeted on reducing pro-inflammatory cytokines such as TNF-α blockers (Berthold-Losleben et al., 2009; Bou Khalil et al., 2011), which are readily available for the treatment of inflammatory diseases (e.g., Aaltonen et al., 2012). However, carefully designed randomised controlled clinical trials will be required to investigate the role of cytokines as a treatment target in AN.

4.4. Methodological considerations

Several methodological issues, including technical factors and clinical confounders, may contribute to the moderate to high heterogeneity observed. Most studies did not account for confounding clinical and lifestyle factors that have been shown to affect cytokine production, e.g., age, menstruation, smoking status, medication, exercise, body fat, and concurrent diagnoses relating to physical and mental health (Dugué et al., 1996; Goebel et al., 2000; Haack et al., 1999; Munzer et al., 2013; Ouchi et al., 2011; Rom et al., 2013). Thus, future studies need to consider factors that may influence the measurement of cytokine concentrations within their study design and analyses.

Few studies measured or reported relevant clinical characteristics such as illness duration, age of illness onset, or ED symptom severity. Variability in such sample characteristics may contribute to the observed heterogeneity. In addition, participants' treatment status varied and differences associated with these, such as the opportunity to engage in ED behaviours (e.g., restrictive eating, purging), may impact on cytokine concentrations (e.g., Canavan et al., 2005). Of interest, research in other psychiatric disorders has shown that certain clinical characteristics are associated with cytokine levels (e.g., Dunjic-Kostic et al., 2013a; Dunjic-Kostic et al., 2013b; Gill et al., 2008). Therefore, future studies would benefit from including information on such clinical factors to further determine the role of cytokines in EDs.

With regards to the HC samples, not all studies used a validated measure to systematically screen and exclude for current or previous psychiatric disorders in the HCs. This cannot rule out the presence of other psychiatric disorders, which as previously cited, have been shown to impact cytokine concentrations. Biases such as these in the HC sample may influence the accuracy of the results.

An additional consideration is that the specific methodologies in each of the laboratories used to measure cytokine concentrations is likely to vary considerably between studies, including the

equipment used (e.g., immunoassays, bioassays). Different assay procedures may yield different results (Zhou et al., 2010) and certain platforms for cytokine assessment are more sensitive than others (Malekzadeh et al., 2017). However, use of random effects models accounts for such between-study heterogeneity.

4.5. Strengths and limitations

The primary strength of this meta-analysis was its objectivity and the systematic evaluation of cytokine concentrations across multiple EDs, utilising all available research to date in this area. This meta-analysis was also conducted and reported in line with the PRISMA guidelines. In addition, a number of methodological considerations have been highlighted, which can be incorporated into future studies in order to advance our understanding of immunological factors involved in the pathophysiology of EDs. We have expanded on the previous meta-analysis of cytokines and AN (Solmi et al., 2015) by including all EDs, identifying 4 new studies assessing cytokines in AN participants, and also applying additional data from 2 previously included studies (Corcos et al., 2001; Vaisman et al., 2004) to provide a more comprehensive analysis. All analyses, except for the assessment of TNF-α in BN and TGF-β in AN, showed no evidence of publication bias.

With regards to limitations, few studies assessed concentrations of TGF-β and also more generally, cytokines in BN and AN-BP. It has been suggested that a minimum of 5 studies is required in order to achieve reasonable power for a random effects meta-analysis, which is greater than the power from the individual studies (Jackson and Turner, 2017). Therefore, the meta-analyses of TGF-β and these patient groups may lack power to detect an effect and the current results should be interpreted with caution. Furthermore, as associations between binge eating and purging symptoms and TNF-α concentrations have been identified (Lofrano-Prado et al., 2011), future studies should aim to expand the literature base of cytokines and BN. This also warrants the measurement of cytokine concentrations in individuals diagnosed with BED, for which no data are currently available. In comparison to Solmi et al. (2015), we limited our research question to cytokines only and did not include data on the associated receptors. To provide a more full and complex picture of the cytokine network, future reviews should consider the role of cytokine receptors across EDs.

The predictive capability of the meta-regressions was limited due to missing data on covariates of interest (e.g., BMI and illness duration) in several studies. While many longitudinal studies were eligible for inclusion, meta-analyses could not be conducted as the data needed to control for within-group correlations between time points were not available. As inflammatory processes, and in particular cytokines, have been suggested as a possible biomarker involved in illness staging and the neuroprogression of illness (McGorry et al., 2014), the opportunity to include longitudinal data may have provided greater insight into potential biological mechanisms underlying EDs and whether cytokine concentrations could be a marker of treatment response.

4.6. Conclusions and future directions

Current treatments for people with EDs are limited, and an improved understanding of the underlying biology may lead to novel treatment strategies. The current meta-analysis found that participants with EDs showed elevated concentrations of circulating TNF-α and IL-6, but not IL-1β or TGF-β, compared to HCs. This pattern of results was also observed in AN, but not BN participants when analyses were stratified by ED diagnosis. The majority of the meta-analyses showed moderate to high heterogeneity, which could be accounted for by various methodological issues, such as limited measurement and/or reporting of confounding factors in the measurement of cytokines (e.g., smoking status), variability in equipment and methods used to measure cytokines, and heterogeneity in sample characteristics.

Further studies should measure a broad range of cytokines and given that cytokines are part of a complex network, they should be analysed in functionally meaningful cytokine groupings. Furthermore, future research should consider measuring cytokines in all EDs, including BN and BED, and include separate analyses of AN subtypes. This research would also benefit from including psychological assessments of ED symptoms alongside cytokine measurement. This will permit further

assessment of the role of cytokines in EDs and may provide the basis for investigations into immunomodulatory medication as a treatment for EDs.

Figure Legends

Figure 1. PRISMA flow diagram. Abbreviations: DSM = Diagnostic and Statistical Manual of Mental Disorders; ICD = International Classification of Diseases.

Figure 2. Forest plot of standardised mean difference in IL-6 between AN participants and HCs from n=12 studies (n=276 AN, n=244 HC). Zero is the line of no effect, and points to the right of zero indicate an elevation in IL-6 in AN compared to HCs. IL-6 was found to be significantly higher in AN participants than in HCs (p=0.001). Abbreviations: SMD = standardised mean difference; CI = confidence intervals.

Figure 3. Forest plot of standardised mean difference in TNF-α between AN participants and HCs from n=17 studies (n=380 AN, n=324 HC). Zero is the line of no effect, and points to the right of zero indicate an elevation in TNF-α in AN compared to HCs. TNF-α was shown to be significantly higher in AN participants than in HCs (p=0.015). Abbreviations: SMD = standardised mean difference; CI = confidence intervals.

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Table 1. Study and sample characteristics for studies included in the meta-analyses.

Study/country		Study design	Sample	N	Mean ± SD age (years)	Mean ± SD BMI (kg/m^2)	Mean illness duration (years)	Diagnostic criteria	ED participants medication status	Parameters of interest	Measurement method
Agnello et al. Italy	2012	Cross-sectional	Female AN Female HC	39 25	26.0 ± 9.0 26.0 ± 3.0	13.9 ± 2 21.0 ± 2	NR -	DSM-IV	NR	TNF-α	Serum - double-antibody ELISA
Ahren-Moonga et al. Sweden	2011	Cross-sectional	Female ED - AN - BN Female HC	26 15 11 12	27.9 ± 8.0 NR NR 28.2 ± 7.2	NR NR NR NR	NR NR NR -	DSM-IV	n=16 antidepressants and/or sedatives	IL-6, TNF-α	Serum - high-sensitivity immunoassay
Allende et al. Spain	1998	Cross-sectional & longitudinal	Female AN BMI<17.5 Female AN BMI>17.5 Female HC	21 19 14	16.9 ± 2.9 16.7 ± 1.6 15.7 ± 1.2	15.8 ± 1.3 20.0 ± 1.9 20.6 ± 1.5	NR NR -	DSM-IV	Medication-free	IFN-γ, IL-1β, IL-2, IL-5, IL-10, TNF-α	Serum - ELISA
Brambilla et al. Italy	1998	Cross-sectional & longitudinal	Female AN-R Female AN-BP Female BN Female HC	9 17 24 26	25.0 ± 8.8 23.3 ± 4.7 23.6 ± 4.4 24.1 ± 3.2	14.8 ± 1.8 17.3 ± 1.8 22.8 ± 2.5 21.7 ± 1.2	6.7 ± 8.5 6.2 ± 3.8 4.2 ± 3.2 -	DSM-IV	Medication-free	IL-1β, IL-6, TNF-α	Plasma - immunoradiometric assays
Brambilla et al. Italy	2001	Cross-sectional	Female AN-R Female AN-BP Female HC	9 5 13	24.5 ± 6.4 19.2 ± 3.1 26.1 ± 1.9	13.4 ± 1.2 14.9 ± 2.3 20.9 ± 1.7	6.2 ± 4.7 2.6 ± 1.8 -	DSM-IV	Medication-free	IL-1β, IL-6, TNF-α,	Plasma - radioimmunoassay
Corcos et al. France	2001	Cross-sectional	Female AN Female HC	29 20	20.1 ± 10.2 23.7 ± 12.1	NR NR	NR -	DSM-IV	NR	IFN-γ, IL-1, IL-2, IL-4, IL-6, IL-10, TGF-β, TNF-α	Serum - ELISA sandwich-type
Dolezalova et al. Czech Republic	2007	Cross-sectional	Female AN Female HC	12 18	NR NR	16.37 ± 1.4 22.96 ± 2.8	NR -	DSM-IV	Medication-free	IL-6	Serum - human serum adipokine LINCOplex kit
Jiskra et al. Czech Republic	2000	Cross-sectional	Female AN-R Female HC	16 16	NR NR	15.0 ± 2.3 22.2 ± 2.5	NR -	DSM-IV	NR	TNF-α	Serum - ELISA
Karczewska-Kupczewska et al. Poland	2012	Cross-sectional	Female AN-R Female HC	20 28	22.3 ± 4.6 25.3 ± 4.9	15.7 ± 1.5 21.3 ± 1.9	1.4 ± 1.0 -	DSM-IV	Medication-free	TNF-α	Serum - immunoassay

Karczewska-Kupczewska et al. Poland	2013	Cross-sectional	Female AN-R Female HC	19 27	22.0 ± 4.8 23.5 ± 3.6	15.9 ± 1.2 22.0 ± 1.9	NR -	DSM-IV	Medication-free	IL-6	Serum - immunoenzymatic method
Krizova et al. Czech Republic	2002	Cross-sectional & longitudinal	Female AN-R Female HC	15 15	NR NR	14.1 ± 6.2 22.5 ± 9.7	NR -	DSM-IV	NR	TNF- α	Serum - ELISA
Krizova et al. Czech Republic	2008	Cross-sectional	Female AN Female HC	28 38	NR NR	15.7 ± 1.9 22.3 ± 2.5	NR -	DSM-IV	NR	TNF-α	Serum - ELISA
Misra et al. United States of America	2006	Cross-sectional & longitudinal	Female AN-R Female HC	23 20	16.2 ± 1.6 15.4 ± 1.8	16.7 ± 1.2 21.9 ± 3.6	0.7 ± 0.9 -	DSM-IV	NR	IL-6	Serum - high-sensitivity sandwich enzyme immunoassay
Nagata et al. Japan	2006	Cross-sectional & longitudinal	Female BN Female HC	20 14	23.1 ± 3.9 24.9 ± 3.5	19.7 ± 2.6 19.8 ± 0.8	NR -	DSM-IV	Medication-free	IL-6	Plasma - ELISA
Nakai et al. Japan	1999	Cross-sectional & longitudinal	Female AN Female HC	20 20	22.1 ± 4.5 20.2 ± 1.3	13.7 ± 1.8 19.9 ± 0.9	3.8 ± 3.6 -	DSM-IV	Medication-free	TNF-α	Plasma - enzyme immunoassay
Nakai et al. Japan	2000	Cross-sectional	Female BN Female HC	20 20	NR NR	NR NR	NR -	DSM-IV	Medication-free	TNF-α	Plasma - enzyme immunoassay
Nogueira et al. France	2010	Cross-sectional	Female AN-R Female AN-BP Female HC	15 9 14	21.1 ± 4.8 25.4 ± 6.9 24.0 ± 2.1	13.3 ± 1.3 13.8 ± 0.9 20.4 ± 1.8	NR NR -	DSM-IV	n=12 medication-free; n=10 antidepressants, neuroleptics and/or anxiolytics	IL-1β, TNF-α	Plasma - ELISA
Ostrowska et al. Poland	2015	Cross-sectional	Female AN Female HC	59 17	15.3 ± 1.6 15.7 ± 1.7	15.3 ± 1.8 20.4 ± 2.2	NR -	DSM-IV	NR	IL-1β, IL-6, TNF-α	Serum - high-sensitivity human ELISA
Ostrowska et al. Poland	2016	Cross-sectional	Female AN-R Female HC	60 20	15.3 ± 1.6 15.7 ± 1.7	15.3 ± 1.8 20.4 ± 2.2	1.0 ± NR	DSM-IV	NR	TGF-β	Serum - ELISA
Pomeroy et al. United States of America	1994	Cross-sectional & longitudinal	Female AN Female HC	16 11	23.3 ± 2.0 27.7 ± 6.6	NR NR	NR -	DSM-III	Medication-free	IL-6, TGF-β, TNF-α	Serum - ELISA (TNF-α) & bioassay (IL-6)
Shimizu et al. Japan	2005	Cross-sectional & longitudinal	Female AN-R Female HC	12 12	13.9 ± 1.1 13.7 ± 1.1	13.9 ± 2.1 17.7 ± 2.0	NR -	DSM-IV	Medication-free	TNF-α	Plasma - ELISA
Terra et al. Spain	2013	Cross-sectional	Female AN-R Female HC	28 33	27.4 ± 1.4 32.6 ± 1.3	16.8 ± 0.2 21.8 ± 0.3	8.3 ± 1.4	DSM-IV	NR	IL-6	Plasma - ELISA

Vaisman et al. Israel	2004	Cross-sectional & longitudinal	Female AN Female HC	7 7	NR NR	NR NR	NR -	DSM-IV	NR	IL-1β, IL-3, IL-6, TNF-α	Serum - solid phase ELISA
Victor et al. Spain	2015	Cross-sectional	Female AN Female HC	24 36	22.4 ± 6.8 24.3 ± 3.4	16.3 ± 1.6 20.9 ± 1.4	NR -	DSM-IV	NR	IL-6, TNF-α	Serum - Luminex 200 flow analyser device
Yasuhara et al. Japan	2007	Cross-sectional & longitudinal	Female AN-R Female HC	7 11	23.2 ±7.8 23.0 ± 2.2	13.6 ± 1.8 19.8 ± 1.8	3.9 ± 4.2 -	DSM-IV	NR	IL-1β, IL-6, TNF-α	Serum - high sensitivity assay

N= number; SD = standard deviation; BMI = body mass index; ED = eating disorder; AN = anorexia nervosa; HC = healthy control; NR = not reported; BN = bulimia nervosa; AN-R = anorexia nervosa restricting subtype; AN-BP = anorexia nervosa binge-purge subtype; DSM = Diagnostic and Statistical Manual of Mental Disorders; ELISA = enzyme-linked immunosorbent assay; TNF = tumor necrosis factor; IL = interleukin; TGF = transforming growth factor; IFN = interferon

Table 2. Summary of comparative outcomes and heterogeneity for all conducted meta-analyses.

	TNF-α						IL-1β						IL-6						TGF-β					
Group	**N (ED, HC)**	**SMD**	**95% CI**	**Z**	***p***	**Heterogeneity**	**N (ED, HC)**	**SMD**	**95% CI**	**Z**	***p***	**Heterogeneity**	**N (ED, HC)**	**SMD**	**95% CI**	**Z**	***p***	**Heterogeneity**	**N (ED, HC)**	**SMD**	**95% CI**	**Z**	***p***	**Heterogeneity**
All EDs combined	798 (454, 344)	0.56	0.17, 0.94	2.80	0.005	I^2 = 84.2%; X^2 = 107.79; df = 17; *p*<0.00	307 (205, 102)	0.77	-0.13, 1.66	1.68	0.093	I^2 = 90.8%; X^2 = 65.22; df = 6; *p*<0.00	589 (331, 258)	0.53	0.19, 0.87	3.08	0.002	I^2 = 72.9%; X^2 = 44.34; df = 12; *p*<0.00	156 (105, 51)	-0.59	-2.37, 1.20	0.65	0.518	I^2 = 95.3%; X^2 = 42.29; df = 2; *p*<0.00
AN	704 (380, 324)	0.48	0.09, 0.87	2.43	0.015	I^2 = 82.8%; X^2 = 92.96; df = 16; *p*<0.00	264 (162, 102)	0.78	-0.17, 1.72	1.61	0.110	I^2 = 91.0%; X^2 = 66.46; df = 6; *p*<0.00	520 (276, 244)	0.59	0.24, 0.94	3.31	0.001	I^2 = 71.6%; X^2 = 38.78; df = 11; *p*<0.00	156 (45, 31)	-0.59	-2.37, 1.20	0.65	0.518	I^2 = 95.3%; X^2 = 42.29; df = 2; *p*<0.00
AN-R	242 (107, 135)	0.12	-0.04, 0.44	0.72	0.470	I^2 = 31.9%; X^2 = 10.27; df = 7; *p*=0.174	108 (44, 64)	0.49	0.08, 0.89	2.37	0.018	I^2 = 0.0%; X^2 = 0.50; df = 3; *p*=0.919	229 (99, 130)	0.36	-0.07, 0.78	1.62	0.105	I^2 = 57.8%; X^2 = 111.84; df = 5; *p*=0.037	-	-	-	-	-	-
AN-BP	84 (31, 84)	-0.50	-1.31, 0.30	1.22	0.221	I^2 = 63.4%; X^2 = 5.32; df = 2; *p*=0.070	84 (31, 84)	1.13	-0.30, 2.57	1.55	0.122	I^2 = 85.4%; X^2 = 13.74; df = 2; *p*=0.001	-	-	-	-	-	-	-	-	-	-	-	-
BN	107 (55, 52)	0.90	-0.26, 2.06	1.52	0.130	I^2 = 87.2%; X^2 = 15.67; df = 2; *p*<0.00	-	-	-	-	-	-	107 (55, 52)	0.13	-0.49, 0.76	0.42	0.67	I^2 = 58.8%; X^2 = 4.86; df = 2; *p*=0.088	-	-	-	-	-	-

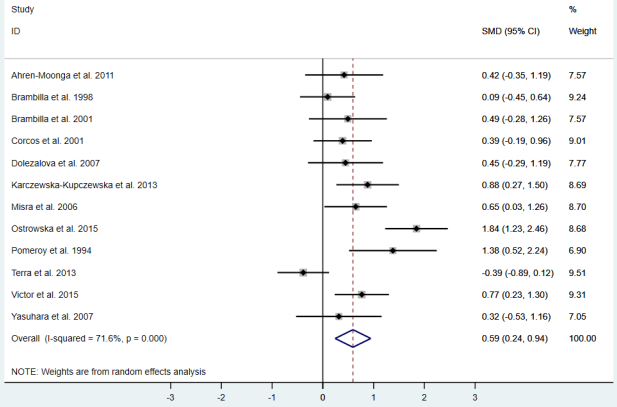
TNF-α = tumor necrosis factor-alpha; IL-1β = interleukin-1 beta; IL-6 = interleukin-6; TGF-β = transforming growth factor-beta; N = number; ED = eating disorder; HC = healthy control; SMD = standardised mean difference; CI = confidence intervals; Z = z score; *p* = *p* value; df = degrees of freedom; AN = anorexia nervosa; AN-R = anorexia nervosa restrictive subtype; AN-BP = anorexia nervosa binge-purge subtype; BN = bulimia nervosa.

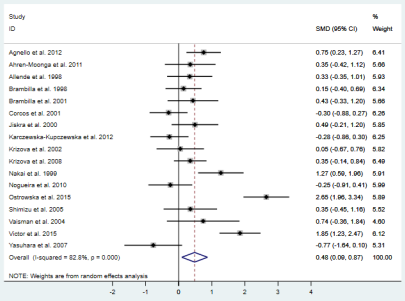
Table 3. Results of the conducted meta-regressions.

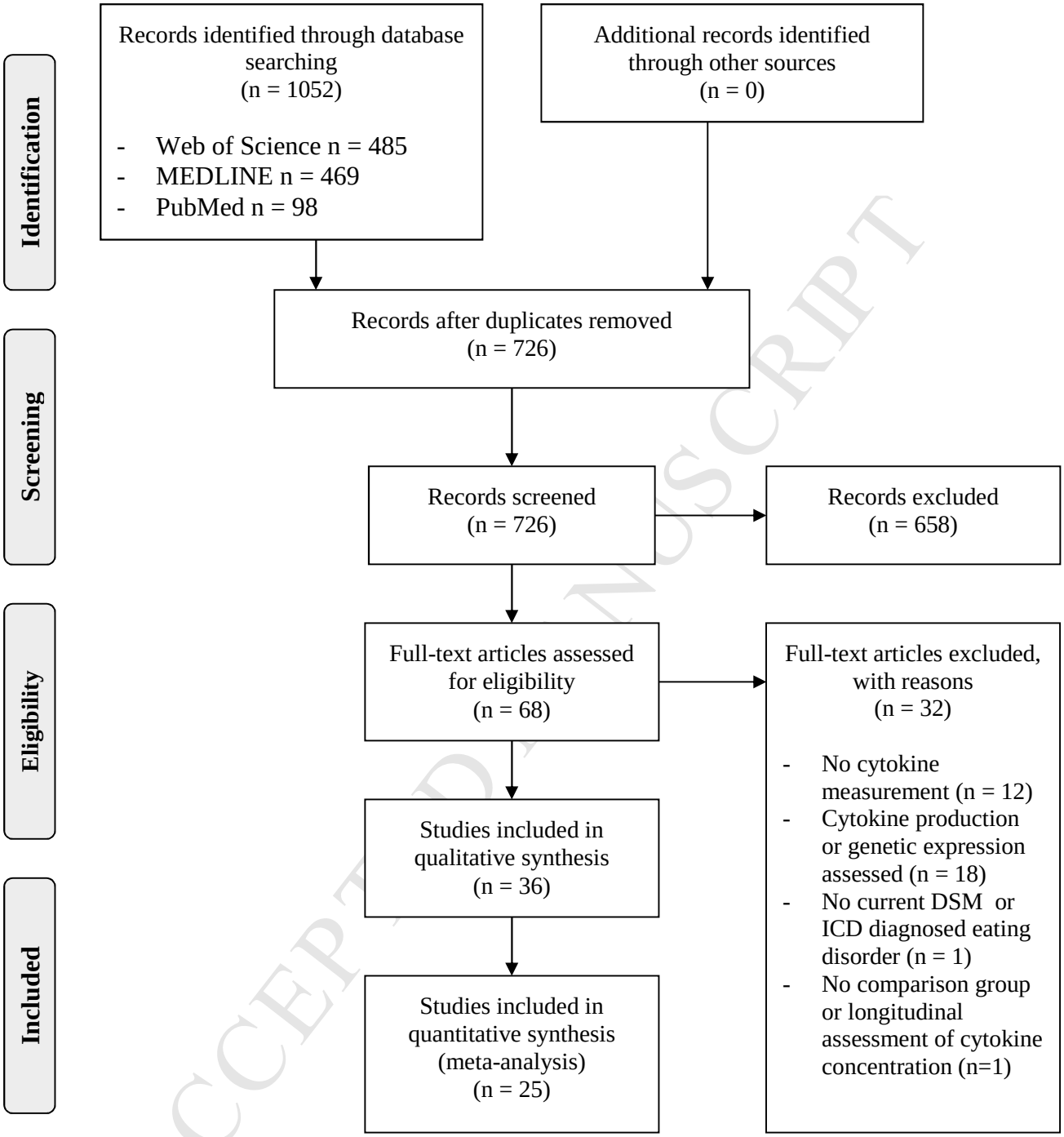
	Covariate	N included studies	Coefficient	L 95% CI	U 95% CI	*p*
Eating disorders studies						
TNF-α	Age	11	-0.083	-0.284	0.119	0.373
	BMI		0.003	-0.384	0.390	0.986
IL-1β	Age	6	-0.279	-0.712	0.154	0.132
	BMI		-0.053	-0.683	0.576	0.805
IL-6	Age	9	-0.132	-0.215	-0.049	**0.008**
	BMI		-0.106	-0.267	0.055	0.157
Anorexia nervosa studies						
TNF-α	Age	11	-0.080	-0.274	0.114	0.369
	BMI		0.204	-0.434	0.842	0.482
IL-1β	Age	6	-0.301	-0.701	0.098	0.096
	BMI		-0.017	-1.189	1.15	0.966
IL-6	Age	8	-0.135	-0.230	-0.039	**0.015**
	BMI		-0.109	-0.460	0.242	0.461

*Significant findings at $p<0.05$ highlighted in bold.

N = number; L = lower; U = upper; CI = confidence interval, *p* = *p* value; TNF-α = tumor necrosis factor-alpha; IL-1β = interleukin-1 beta; IL-6 = interleukin-6; BMI = body mass index







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